

## **REMARKS**

Claims 1-12, 14, 18, and 21 are pending in the application. Claims 1, 4, 5, 10, 11 and 19 are hereby amended and claims 3, 17 and 19-20 are canceled without prejudice or disclaimer, Applicant reserving the right to pursue the subject matter of the canceled claims in a continuation application at a later date. Claims 13, 15, and 16 were previously canceled without prejudice or disclaimer as being drawn to a non-elected invention.

### **Claim Rejections**

#### **35 USC § 112, first paragraph**

Claims 1-12, 14 and 17-20 stand rejected under 35 USC § 112, first paragraph, as not enabled. However, in the Office Action, the Examiner suggested language which would be considered enabled, namely "cultivation of chondrocytes" and "wherein said high extracellular concentrations of Mg range up to 20 mM". Claim 1 has hereby been amended to recite the language suggested by Examiner, thereby overcoming this rejection. Consistent with this amendment, claim 17, which recited that the cells were chondrocytes, is hereby canceled. In addition, claims 4, 5, 10 and 19 have been amended to recite "chondrocytes" instead of "cells", in order to accord with amended claim 1. Claims 3, 19 and 20 have been canceled. Also, claim 1 has been amended to further highlight that at least once during cultivation the unphysiologically high Mg concentration is increased to an even higher unphysiologically high Mg concentration.

In view of the foregoing, reconsideration and withdrawal of this rejection are respectfully requested.

#### **35 USC § 112, second paragraph**

Claims 1-12, 14 and 17-20 stand rejected under 35 USC § 112, first paragraph, as indefinite due to the recitation of "the unphysiologically high extracellular Mg concentration" since this phrase has no antecedent basis. Claim 1 has hereby been amended to recite "an unphysiologically high extracellular Mg concentration", thereby overcoming this rejection.

Claims 6-7 stand rejected under 35 USC § 112, first paragraph, as indefinite due to the recitation of "chondrocytes". Claims 6 and 7 are hereby amended to recite "said" chondrocytes, referring back to the chondrocytes now recited in amended claim 1. Claims 6 and 7 have been further amended to recite more standard English ("are isolated from", claim 6, or "are differentiated from", claim 7), and to remove the unnecessary recitation of "are cultivated" from the claim. Applicant submits that this amendment does not add any new

matter, being entirely formal in nature, and respectfully requests reconsideration and withdrawal of these rejections.

**Rejections under 35 USC § 103(a)**

*Caruso and Egerbacher*

Claims 1-2, 5 and 7-9 stand rejected under 35 USC § 103(a) as obvious over a combination of Caruso (US 4,978,661) and Egerbacher et al (2001; previously cited; hereinafter “Egerbacher”). This rejection is traversed.

The Invention as Claimed

An important feature of the invention lies in the different stages of cultivation, namely in a first stage of cell cultivation at an unphysiologically high extracellular concentration of magnesium and, then at least one subsequent stage of cultivation during which the concentration of Mg is increased to an even higher level of unphysiologically high extracellular magnesium concentration. The effect of the additional raising step during cultivation is discussed in the Examples (see page 13, at lines 35 et seq.). While in the first stage of cultivation, the cells are already at an unphysiologically high extracellular magnesium concentration, and they proliferate. After further raising the magnesium concentration to an even higher level, differentiation of the proliferated cells occurs, thus allowing the cells to become chondrons. Claim 1 has been amended to highlight this feature.

This feature of the invention is reflected in Claim 1, which recites a method for the generation of chondrons and requires a step of cultivating chondrocytes at unphysiologically high extracellular concentrations of Mg, the high extracellular concentrations of Mg ranging up to 20 mM; AND, Claim 1 further requires that at least once during the step of cultivation at a first unphysiologically high extracellular Mg concentration is increased to a second unphysiologically high extracellular Mg concentration. This feature is neither taught or suggested by any of the references cited by the Examiner.

As described by the Examiner on page 9, 2<sup>nd</sup> paragraph of the Office Action, Caruso teaches only the use of 6-halo-quinolone compounds for the treatment of rheumatoid arthritis, and is completely silent concerning any usage of magnesium salts for cultivating chondrocytes for proliferation and differentiation of the same. Applicant notes that Caruso is also completely silent regarding a cultivating step which requires that at least once during cultivation, an unphysiologically high extracellular Mg concentration is increased.

The defects of Caruso as a reference are not overcome or mitigated in any way by Egerbacher. As stated in the previous response, Egerbacher teaches the use of a single level of

Mg, and that is taught as used only with quinolone-treated horse and dog chondrocytes. Egerbacher does not teach that cells which are cultivated at unphysiologically high extracellular concentrations of magnesium, should, during cultivation, be subjected at least once to a change from an original (or first) unphysiologically high concentration to an even higher (or second) unphysiologically high concentration of Mg concentration e.g., from 5mM to 10mM Mg. That is, while Egerbacher does teach cultivation at unphysiologically high extracellular concentration of magnesium (albeit only when quinolone is also used), Egerbacher wholly lacks any teaching or suggestion of an additional increasing step to convert the cells from the proliferation phase to the differentiation phase as demonstrated by the Examples of the present patent application, and as required in claim 1. In view of the requirement in claim 1 that the method is "characterized in that at least once the unphysiologically high extracellular Mg concentration is increased during cell cultivation" (emphasis added), and in view of both Caruso and Egerbacher lacking this feature, Applicant respectfully submits that no combination of Caruso and Egerbacher renders obvious the subject matter of claims 1-2, 5 and 7-9, and respectfully requests reconsideration and withdrawal of this rejection.

*Masuda, Egerbacher, Halvorsen and Lindenberg*

Claims 1-2, 4-9, 14 and 18 stand rejected under 35 USC § 103(a) as obvious over a combination of Caruso (as above), Egerbacher et al (as above), Halvorsen (US 6,841,150, previously cited) and Lindenberg et al., (US 2005/0239040, hereinafter "Lindenberg"). This rejection is also traversed.

The deficiencies of Caruso and Egerbacher are described above. Briefly, among other defects, neither reference teaches the cultivation of chondrocytes in higher than physiologically normal extracellular Mg concentrations, in which at least once during the cultivation, an extracellular Mg concentration is changed to an even higher extracellular Mg concentration.

Halvorsen teaches a method for directing adipose-derived stromal cells cultivated *in vitro* (e.g. in calcium alginate) to differentiate into functional chondrocytes. However, the method of Halvorsen differs substantially from the present method and teaches only conditions such temperatures between 31 and 37 °C in a humidified incubator, CO<sub>2</sub> content between 2 and 10%, and O<sub>2</sub> between 1 and 22%. Halvorsen provides no teaching whatsoever of the significance of using higher than physiologically normal extracellular Mg concentrations, and in particular there is no teaching of shifting such a high extracellular Mg

concentration to a still higher concentration during incubation, in order to promote chondrocyte differentiation. Halvorsen does supply or make obvious this feature, and does not cure or mitigate the deficiencies of Caruso and Egerbacher in any way.

Lindenberg is even less relevant in that Lindenberg's teachings are related to a totally different area of biology. Lindenberg's methods and teachings relate to *in vitro* fertilization, and, as stated by the Examiner, the conditions described by Lindenberg apply to the *in vitro* culture of oocytes. Chondrocytes are not mentioned at all by Lindenberg. Mg is also not mentioned by Lindenberg. Applicant submits that one of skill in the art would not seek to apply such unrelated teachings to the art of chondrocyte differentiation. Lindenberg does not deal with chondrocytes or with Mg concentrations in any way, let alone suggest a change of extracellular Mg concentrations, already at a level that is higher than physiologically normal, to a yet higher level during cultivation of any type of cell whatsoever. Thus, the deficiencies of Caruso and Egerbacher are neither cured nor mitigated in any way by the teaching of Lindenberg.

In view of the foregoing, Applicant respectfully requests reconsideration and withdrawal of this rejection.

#### **Concluding Remarks**

In view of the above, it is respectfully requested that the application be reconsidered, that claims 1-12, 14, 18 and 21 be allowed, and that the application be passed to issue.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at the local telephone number listed below to discuss any other changes deemed necessary in a telephonic or personal interview.

A provisional petition is hereby made for any extension of time necessary for the continued pendency during the life of this application. Please charge any fees for such provisional petition and any deficiencies in fees and credit any overpayment of fees to Attorney's Deposit Account No. 50-2041.

Respectfully submitted,



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